PRELIMINARY AMENDMENT
NATIONAL STAGE ENTRY
OF PCT/EP03/08305

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Original) A recombination process for recombining a population of heterologous polynucleotides comprising variant groups and/or regions, wherein recombination is performed within one group of polynucleotides and/or between defined regions of polynucleotides by using different restriction sites in different groups and/or regions of polynucleotides.

Claim 2. (Currently Amended) A recombination process according to claim 1,

- wherein the polynucleotides comprise at least one section defining a structural unit and at least one section defining an interstructural motif;

- wherein in the population at least two variant interstructural motifs are present; and

- wherein variant interstructural motifs are recombined separately by using different restriction sites in different interstructural motifs.

Claim 3. (Currently Amended) A recombination process for recombining at least two polynucleotides comprising at least a structural unit and at least one interstructural motif, comprising:

- cleaving the polynucleotides with at least one restriction enzyme creating a non-palindromic cohesive end thus creating a recombination site;
- wherein the recombination site is present in the interstructural motif which comprises a coding region of the polynucleotide; and
- ligating the resulting mixture of fragments.

Claim 4. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, characterised in that polynucleotides are recombined wherein the structural unit of different polynucleotides are heterologous and wherein the structural units are optionally made of structural subunits which are connected by interstructural motifs.

Claim 5. (Original) A recombination process according to claim 3, wherein the structural units and/or the structural subunits are recombined.

Claim 6. (Currently Amended) A recombination process according to claim 2~~, 3 or 4~~, wherein the interstructural motif comprises a coding region of the polynucleotide, a non-coding region or a part of a vector sequence.

Claim 7. (Currently Amended) A recombination process according to ~~at least one of the above claims~~ claim 1, wherein

at least one restriction enzyme is used which creates a non-palindromic cohesive end.

Claim 8. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 7, wherein the restriction endonuclease creating the non-palindromic cohesive end is a PRE.

Claim 9. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein polynucleotides are recombined which are present in a vector.

Claim 10. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1,

- wherein the polynucleotides are cleaved with at least one restriction enzyme creating non-palindromic cohesive ends,
- wherein the cleaved vectors are ligated at high DNA concentrations allowing the formation of concatemers,
- wherein the concatemers are resolved by cleavage with at least one further restriction enzyme, and
- wherein the vector constructs are recircularised by ligation.

Claim 11. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 2, wherein for different interstructural motifs different restriction enzymes are used thus creating unique sites for recombination.

Claim 12. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 2, wherein for different interstructural motifs the same restriction enzymes is used wherein the nucleotides are different at the

cleavage site in different interstructural motifs thus creating unique restriction sites for recombination.

Claim 13. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein a resolving enzyme is used which creates cohesive ends.

Claim 14. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein a resolving enzyme is used which creates non-palindromic cohesive ends.

Claim 15. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 13, wherein the restriction site for the resolving enzyme is present in the interstructural motif.

Claim 16. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein the binding sites for one or more restriction enzymes and/or the nucleotide variation at the cleavage site are introduced by site specific mutation at predefined positions.

Claim 17. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein the polynucleotides are vector constructs comprising sequence sections defining at least two structural units.

Claim 18. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein the polynucleotides comprise a variant gene family.

Claim 19. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1,

- wherein the polynucleotides comprise a section encoding at least a variable heavy fragment of an antibody and/or a section encoding at least a variable light fragment of an antibody.

Claim 20. (Currently Amended) A recombination process according to ~~at least one of the above claims~~claim 1, wherein a population of polynucleotides is recombined wherein the polynucleotides encode at least the variable fragment of an antibody which defines the structural unit,

wherein the CDR regions of the variable fragment define the structural subunits that are recombined with each other and at least the FRs define the interstructural motifs,

wherein at least two different subclasses of the variable fragments are encoded by the polynucleotide population thus defining different groups, and

wherein recombination is performed within each subclass separately by using for each subclass a different restriction site in the interstructural motif.

Claim 21. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein the variable heavy fragment comprises the VH and CH1 domains and the and variable light fragments comprise the VL and the CL domains.

Claim 22. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein the different framework subclasses present in the population of

polynucleotides which are recombined are cleaved with different restriction enzymes creating cohesive ends.

Claim 23. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein the restriction site is present within the FR3.

Claim 24. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein the polynucleotides encode the variable heavy and the variable light fragment of an antibody.

Claim 25. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein at least one additional recombination site is located between the variable heavy and the variable light fragment which is used for shuffling the variable heavy fragment against the variable light fragment.

Claim 26. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein a restriction endonuclease is used for shuffling the variable heavy fragment against the variable light fragment, which creates non-palindromic cohesive ends.

Claim 27. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein the polynucleotides are vector constructs which comprise sections encoding the variable heavy and the variable light fragment of an antibody, wherein at least two different subclasses of the

variable fragments (heavy and/or light fragment) are encoded by the polynucleotide population thus defining different groups,

- wherein the vectors are cleaved with at least one restriction enzyme creating a non-palindromic cohesive end at a restriction site present in-between the sections encoding the variable heavy and the variable light fragment of an antibody thus allowing recombination between the heavy and the light fragments;

- wherein upon usage of the at least one restriction enzyme a different cohesive end site is created in each group;

- wherein the fragments are ligated thus allowing the formation of concatemers containing only members of one group; and

- wherein the concatemers are resolved by the use of at least one restriction enzyme at a restriction site present at an interstructural motif.

Claim 28. (Currently Amended) A process wherein, for resolving of the concatemers different restriction enzymes are used for different subclasses.

Claim 29. (Currently Amended) A process according to ~~at least one of the above claims~~claim 20, wherein a phage and/or phagemid display vector is used as a vector.

Claim 30. (Currently Amended) A method for evolving a molecule by selection and recombination comprising a recombination process according to ~~at least one of the above claims~~claim 20.

Claim 31. (Currently Amended) The method according to claim 30 comprising:

a.) creating a starting library by cloning the polynucleotides to be recombined into an expression vector if the polynucleotide itself is not a vector,

b.) recombining the library members with a process according to ~~at least one of the above claims 1 to 29~~claim 1,

c.) selecting candidates with desired characteristics,

d.) optionally performing further rounds of selection and recombination.

Claim 32. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the library members respective the selected candidates are recombined with themselves or with other selected clones or members of the naive library.

Claim 33. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the polynucleotides are expressed via a method which allows physical coupling of pheno- and genotype.

Claim 34. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the recombined polynucleotides are expressed in a cell and selection is performed by screening or selecting for a particular phenotype amongst the clones.

Claim 35. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the oligonucleotides are selected for physical properties.

Claim 36. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein a display library is created.

Claim 37. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the selection step comprises selection for affinity to a defined target.

Claim 38. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein a phagemid library is created.

Claim 39. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the polynucleotides are mutagenised.

Claim 40. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein further recombination processes are performed in addition to the recombination processes according to at least one of the above claims.

Claim 41. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein the further recombination processes are *in vitro* and/or *in vivo* recombination processes.

Claim 42. (Currently Amended) The method according to ~~at least one of the above claims~~claim 31~~ to 41~~, wherein a selection process is after step a.) and before step b.)~~ a selection procedure is~~.

Claim 43. (Currently Amended) The method according to ~~at least one of the above claims~~claim 30, wherein recombination is

performed at a type IIs restriction site already present in the library.

Claim 44. (Original) A library containing heterologous polynucleotides comprising variant groups and/or variant regions within the polynucleotide, wherein the members of one group and/or the same regions have identical unique restriction sites which differ from the restriction sites present in other groups and/or regions.

Claim 45. (Original) A library according to claim 44, wherein the polynucleotides comprise at least one section defining a structural unit and at least one section defining an interstructural motif, wherein in the population at least two variant interstructural motifs are present.

Claim 46 (Original) A library according to claim 44 or 45, wherein the structural unit of different polynucleotides are heterologous and wherein the structural units are optionally made of structural subunits connected by interstructural motifs.

Claim 47. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the polynucleotides are or are present in a vector.

Claim 48. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the interstructural motif comprises a coding region of the polynucleotide, a non-coding region or part of the vector sequence.

Claim 49. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the unique restriction sites are located at the interstructural motifs.

Claim 50. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein at least one interstructural motif has a binding site for a PRE.

Claim 51. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the polynucleotides are derived from nature and/or are at least partially synthetical polynucleotides sequences.

Claim 52. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the polynucleotides comprise a variant gene family.

Claim 53. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44,

- wherein the polynucleotides comprise a section encoding at least a variable heavy fragment of an antibody and/or a section encoding at least a variable light fragment of an antibody.

Claim 54. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44,

wherein the polynucleotides encode at least the variable fragment of an antibody which defines the structural unit,

wherein the CDR regions of the variable fragment define the structural subunits and at least the FRs define the interstructural motifs,

wherein at least two different subclasses of the variable fragments are encoded by the polynucleotide population thus defining different groups, and

wherein each subclass has a different restriction site in the interstructural motif.

Claim 55. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the variable heavy fragment comprises the VH and CH1 domains and the and variable light fragments comprise the VL and the CL domains.

Claim 56. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the restriction site for a resolving enzyme is present within the FR3 of the heavy chain.

Claim 57. (Currently Amended) A library according to ~~on of the above claims~~claim 44, ~~wherin~~ wherein the restriction site for a resolving enzyme is present within the FR3 the light chain.

Claim 58. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the polynucleotides encode the variable heavy and the variable light fragment of an antibody.

Claim 59. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein at least one additional recombination site is located between the variable heavy and the variable light fragment which is used for shuffling the variable heavy fragment against the variable light fragment.

Claim 60. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the polynucleotides are vector constructs which comprise sections encoding at least the variable heavy and the variable light fragment of an antibody, wherein at least two different subclasses of the variable fragments (heavy and/or light

fragment) are encoded by the polynucleotide population thus defining different groups.

Claim 61. (Currently Amended) A library according to ~~at least one of the above claims~~claim 44, wherein the vector is a phage or phagemid vector.

Claim 62. (Currently Amended) A process for producing a substance with special characteristics, comprising:

- recombining and selecting a polynucleotide library for special characteristics according to ~~at least one of the claims~~claim 44~~ to 60~~ according to a process for recombining a population of heterologous polynucleotides comprising variant groups and/or regions, wherein recombination is performed within one group of polynucleotides and/or between defined regions of polynucleotides by using different restriction sites in different groups and/or regions of polynucleotides~~according to claim 1 to 43~~,

- recovering at least one clone comprising the polynucleotide depicting the desired characteristic,

- amplifying said polynucleotide *in vivo* or *in vitro*,

- manufacturing the substance which is either the expression product of the polynucleotide or comprises the latter.

Claims 63-66. (Cancelled).